FT-MRU990

TCO (Trans-CycloOctyne) reagents for "Click Chemistry" – Amine reactive

Products information

Primary amine reactivity (NHS) –
TCO-NHS Ester reacts specifically and efficiently with a primary amine (e.g., side chain of lysine residues or aminosilane-coated surfaces) at pH 7-9 to form a covalent bond.

Extended PEO spacer – improves labeling efficiency, enhances solubility, and minimizes steric hindrance
The hydrophilic polyethylene glycol (PEG) spacer arm significantly improves labeling efficiency, imparts water solubility, and reduces aggregation of labeled proteins stored in solution. The PEG spacer arm also gives the re agent a long and flexible connection that minimizes steric hindrance involved with ligation to complementary tetrazine-containing molecules.

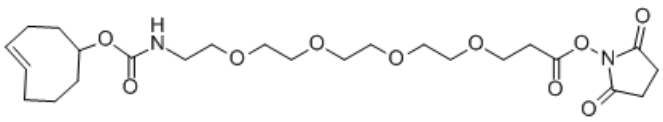
Solubility: Chloroform, DCM, DMF, DMSO
Store at -20°C(M)(ship at RT)

TCO-NHS

An amine-reactive labeling reagents with enhanced solubility in aqueous buffers provided by a PEG4 spacer.

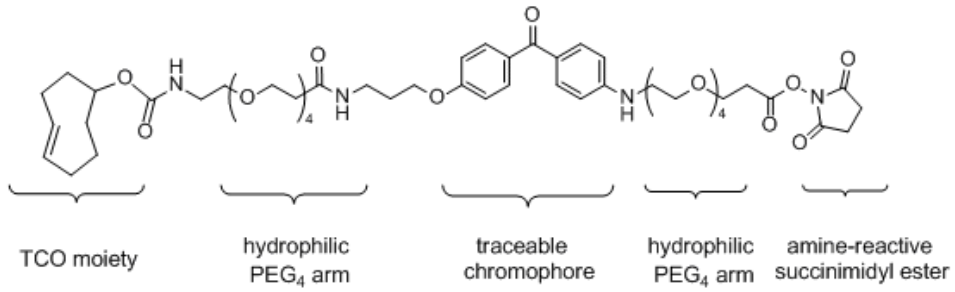
TCO-NHS ester — **MRU260, 25mg /100mg / 1G**
trans-Cyclooctene–NHS ester; MW: 267.28; (M)

TCO-PEO$_4$-NHS ester — **MRU990, 4x2mg, 10mg / 25mg /100mg**
trans-Cyclooctene-PEG$_4$-NHS ester; MW: 514.57; (M). Technical Sheet

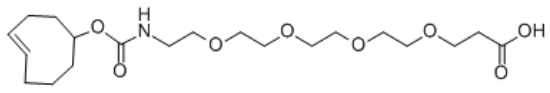


TCO-PEO$_{12}$-NHS ester — **1J4580, 4x2mg 10mg / 25mg /100mg**
trans-Cyclooctene-PEG$_{12}$-NHS ester; MW: 866.99; (M)

UV-tracableTCO-PEO$_{12}$-NHS ester — **MRU230, 4x2mg 10mg / 25mg /100mg**
UV-Tracer™ Trans-Cydooctene-NHS ester; MW: 1015.15 (M)



TCO-PEO$_4$-COOH — **1J4590, 25mg /100mg/500mg**
Trans-Cyclooctene-PEG$_4$-Acid; MW: 417.49 (M)



Chemical Structure of TCO-PEG4-Acid

Crosslinking Biomolecules using Click Reactions

TCO Click Chemistry

Biocompatible – Chemoselective - Efficient - Unprecedented kinetics –

• Selective: Tetrazines and trans-cyclooctene groups react with each other with high efficiency in the presence of other functional groups found in biological samples
• Conjugation efficiency > 99 % under mild buffer conditions without requiring a toxic catalyst (e.g. Cu(I)) or reducing agents (e.g. DTT)
• TCO functional group remains stable in aqueous buffered media (weeks at 4°C, pH 7.5)
• Reactions complete in 30-60 minutes at low protein concentrations (5-10 M)
• By far, the fastest kinetics among any other available bioorthogonal reaction pairs

The inverse-electron demand Diels-Alder cycloaddition reaction of trans-Cyclooctenes (TCO) with tetrazines is a bioorthogonal reaction that possesses exceptional kinetics (k > 800 M-1s-1) and selectivity. Such excellent reaction rate constants are unparalleled by any other bioorthogonal reaction pair described to date.
The extremely fast kinetics and selectivity enables the conjugation of two low abundance biopolymers in an aqueous and otherwise complex chemical environment through the formation of a stable dihydropyridazine. This bioorthogonal reaction possesses extreme selectivity and biocompatibility, such that the complimentary reagents can form covalent bonds within richly functionalized biological systems, in some cases, living organisms. The TCO-tetrazine click reaction is a very powerful tool in catalyst-free protein-protein bioconjugation.

Applications

Applications:
Protein-peptide conjugates
Protein-antibody conjugates
Peptide-small molecule conjugates
18F radiolabelling
Protein-oligonucleotide conjugates
Surface modification

Directions for use

Guidelines for use

Important product information

● NHS esters are moisture-sensitive and readily hydrolyze. Avoid moisture condensation by allowing product to come to room temperature before opening. Prepare working stock solutions immediately before use and discard unused portion.
● Hydrolysis is a competing reaction with primary amines of proteins/peptides. Acylation is favored using concentrated protein solutions (1-5 mg/mL) at pH 7-9. For NHS ester reactions, use an amine-free buffer such as 100 mM sodium phosphate, 150 mM sodium chloride, pH 7.5. Do not use buffers containing primary amines (e.g. Tris, glycine). Prior to use, dissolve the reagent in a dry water-miscible organic solvent such as DMSO or DMF.
● Reactions between tetrazine and TCO are complete in 30-60 minutes at 5-10 µM

Additional Material Required
• Water-miscible organic solvent such as dimethyl sulfoxide (DMSO) or dimethyl formamide (DMF)
• Reaction buffer: Phosphate-buffer (100 mM sodium phosphate,
150 mM NaCl, pH 7.5) or other suitable amine-free buffer at pH7 - 9
• Quenching buffer: 1 M Tris-HCl pH 8.0
• Spin Desalting Colum

Procedure protocol for labeling proteins

1. Buffer exchange proteins into phosphate reaction buffer at 1-5 mg/mL using a desalt spin column.
2. Immediately before use prepare 10 mM TCO-PEG12-NHS reagent in DMSO or DMF.
3. Add a 20-fold molar excess NHS reagent to the protein sample and incubate for 1 hr at room temperature.
4. Stop the reaction by adding Quench Buffer (e.g. 1 M Tris·HCl, pH 8.0) to a final concentration of 50-100 mM; incubate for 5 minutes.
5. Remove excess reagent by desalting the labeled protein through a desalt spin column or by dialysis.

Protein-Protein Tetrazine/TCO Conjugation

• Calculate volume tetrazine-labeled protein (1 - 5 mg/ml) equivalent to a 2 - 5 fold molar excess over desired volume TCO-labeled protein (1 - 5 mg/ml).

• Mix calculated volume tetrazine -labeled protein with desired volume of TCO-labeled protein.

• Allow reaction to proceed for 60 minutes at room temperature.

• Store conjugate at 4°C until ready for purification or use.

General protocol for click reaction

1. Prepare the TCO-containing protein in reaction buffer.
2. Add tetrazine-containing sample to TCO-containing sample.

Recommendation:
Add 1.05-1.5 mol equivalents of tetrazine-PEG reagent to 1 mole equivalent of TCO-containing protein.

3. Incubate the reaction at room temperature or at 40C requires 30 min-2 hours.
4. The reaction is now ready for purification by size exclusion chromatography if required.

Troubleshooting:

Problem	Possible Cause	Solution
No or poor labeling of protein with TCO	NHS-ester hydrolyzed	Allow product to equilibrate to room temperature before opening. Use only high quality, anhydrous water-miscible solvents such as DMSO or DMF
	Amine- contaminants in protein labeling reaction buffer (e.g. glycine, Tris)	Buffer exchange proteins into an amine-free buffer before labeling (e.g. 100 mM sodium phosphate, 150mM sodium chloride, pH 7.5)
	Sub-optimal reaction conditions.	Optimize labeling conditions by altering molar excess

Selected References:

Devaraj et al. (2009) Fast and Sensitive Pre-Targeted Labeling of Cancer Cells through a Tetrazine/trans-Cyclooctene Cycloaddition. Angew. Chem. Int. Ed. 48:7013.
Haun et al. (2009) Probing Intracellular Biomarkers and Mediators of Cell Activation Using Nanosensor and Bioorthogonal Chemistry. ACS Nano. 5:3204.
Blackman et al. (2008) Tetrazine Ligation: Fast Bioconjugation Based on Inverse-Electron-Demand Diels-Alder Reactivity. J. Am. Chem. Soc. 130:13518.
Devaraj et al. (2008) Tetrazine-Based Cycloadditions: Application to Pretargeted Live Cell Imaging. Bioconjugate Chem. 19:2297.

Related / associated products and documents

Click Solvent (DMSO / Tert-Butanol, 3:1) #ZC6950 **Alkyne reagents** ()
DBCO reagents: DBCO – COOH (FT-DQP580), fluorochromes (FT-DQP790), Others

See Product hightlights, Biosciences Innovation and e-search tool.

Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com.

For any information, please ask : Uptima / Interchim; Hotline : +33(0)4 70 03 73 06
Order on-line or Contact your local distributor

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